Daland (J.)

A NEW METHOD OF SEPARATING THE WHITE FROM THE RED BLOOD CORPUSCLES BY MEANS OF THE HÆMATOKRIT.

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[*A lecture delivered before the Franklin Institute, December 9, 1892.*]

The lecturer was introduced by the Secretary of the Institute, and spoke as follows:

MEMBERS OF THE INSTITUTE, LADIES AND GENTLEMEN:

Before referring to the methods of separating the white from the red blood corpuscles, it may be of interest if I mention briefly a few of the more important facts regarding the blood.

This fluid has been the object of study by all physicians at all places, at all time; and this interest has not been confined to physicians, but has exercised its fascination over the remaining professions as well as the general public.

All through literature, both scientific and secular, constant reference is made to this wonderful liquid.

Notwithstanding the centuries that have been devoted to research in this direction but little was known of the exact composition of the blood in health and disease until the early part of the present century, and our knowledge of the existence of the red blood cells is but 220 years old.

The blood may be described as having a bright scarlet-red color in arteries, and dark bluish-red when obtained from a vein; its reaction is neutral alkaline; its odor peculiar but difficult to describe; its taste is saline; its specific gravity averages 1·055 and its temperature varies from 99° to 100° F.

The total quantity of blood is estimated as one-thirteenth of the body weight, *i. e.*, a man weighing 130 pounds contains ten pints of blood.

Broadly speaking, the blood is composed of a fluid called *plasma*, in which floats small solid bodies, known as the *red* and *white* blood corpuscles.

The plasma is a liquid composed of salts and albumin dissolved in water. The red blood cell is a round body four times as broad as it is thick, concave on each side, with rounded edges and measures about $\frac{1}{3000}$ to $\frac{1}{3500}$ of an inch in diameter, and with a tendency to form rouleaux.

When viewed under the microscope it is light yellow in color, which color is due to the presence of blood pigment or hæmaglobin.

The white blood corpuscle is a spherical body, the average size of which is a trifle larger than a red blood corpuscle; it is colorless and is possessed of the curious property of throwing out processes and retracting them, which is called amæboid movement.

Some years ago an attempt was made to count the number of red and white corpuscles in each cubic millimetre of blood, and finally the Thoma-Zeiss blood counter was constructed. The instrument is composed of a capillary tube which opens into an olive-shaped bulb, containing a small glass ball about the size of a large shot. The second portion consists of a glass slide, in the centre of which is an elevated disc surrounded by a gutter. When a glass cover is placed on top a shallow cell is formed, having known dimensions. On the surface of the disc is traced a series of lines, bisecting each other in such a way as to form sixteen small squares.

By aid of the graduated pipette the blood is diluted with a two and one-half per cent. solution of potassium bichromate in the proportion of $\frac{1}{100}$. This is thoroughly mixed in the bulb of the pipette, with the aid of the glass ball, by thorough shaking. A drop of this diluted blood is then placed upon the slide, over which is placed a cover glass. The number of red cells in each small square are then counted. This number is multiplied by the dilution, and

this result again by 4,000, because each square is but the $\frac{1}{4000}$ of a cubic millimetre, and finally this result is divided by the number of small squares counted.

The process proved so uncertain in its results, and requires so much time and patience, that in 1885 Professor Blix first suggested the use of centrifugal force, and later Dr. Hedin perfected this instrument, which I herewith demonstrate.

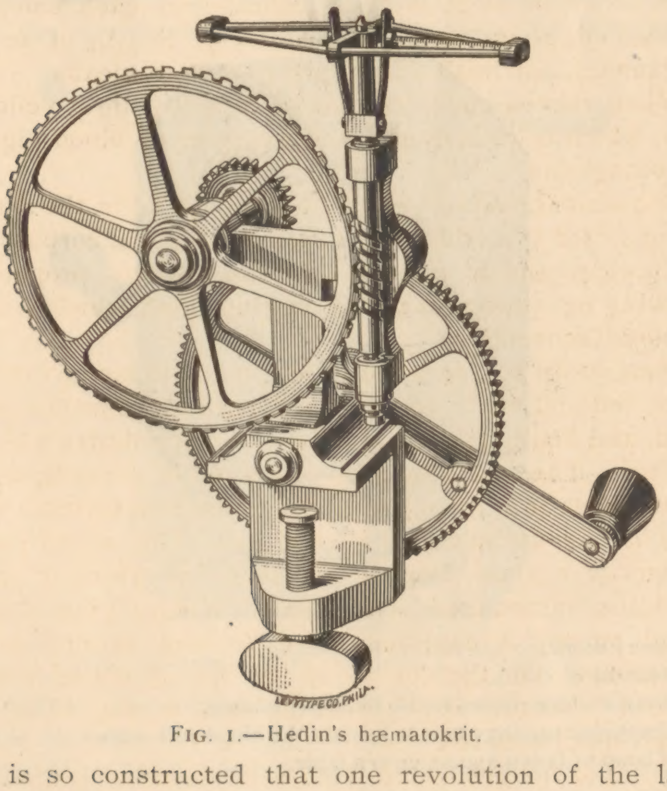

FIG. 1.—Hedin's hæmatokrit.

It is so constructed that one revolution of the large wheel, to which is attached the handle, causes the brass frame at the top of an upright piece of steel to revolve 104 times. This frame is so arranged that it receives two glass tubes, which are to contain the blood, each measuring thirty-five millimetres long, and held securely in position by a spring. The apparatus is extremely simple, compact, durable, and is admirably adapted for the purpose for

which it is intended. The glass tubes, that are to contain the blood to be rotated, have a capacity of 27·489875 cubic millimetres, are 3·75 millimetres thick, 35 millimetres long, and have a calibre throughout measuring one millimetre in diameter. On the outside is a scale dividing it into fifty equal parts, in the same manner as the scale on

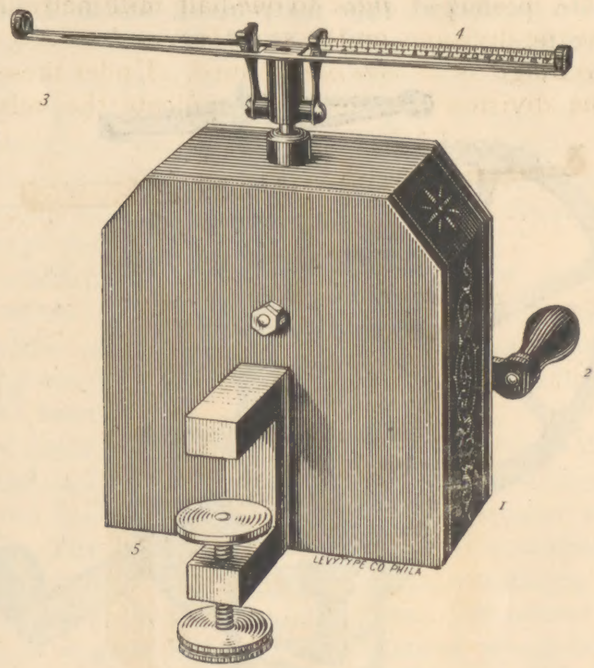

FIG. 2.—Author's hæmatokrit.

1, Box containing mechanism for rotating.
2, Handle of crank.
3, Frame which rotates 10,000 times per minute.
4. Improved capillary tube to contain the blood with scale.
5, Clamp to fasten apparatus to a table.

an ordinary thermometer. The method of determining the volume of corpuscles is very simple: the blood is mixed with an equal quantity of a fluid preventing coagulation, and is then rotated; the red corpuscles form a column at the periphery of the tube, and measure, we will say, twelve and one-half. As the blood is diluted one-half, this result is multiplied by 2 = 25, and to convert this into percentage

it is again multiplied by 2, as the scale is divided into but fifty parts, which gives fifty per cent.

The improved hæmatokrit (see *Figs. 2* and *3*), which I have suggested, and which I exhibit this evening, presents the following advantages:

The tubes (see *Fig. 5*) measure seventy millimetres in length, the lumen is *reduced* to one-half millimetre in diameter, and the divisions on the scale *increased* to 200, so that the percentage is at *once* determined. Under these conditions, the divisions on the scale indicate the volumetric

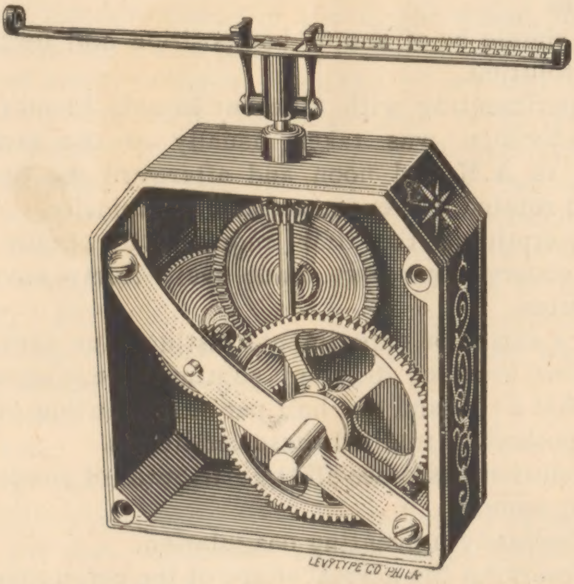

FIG. 3.—Interior view of author's hæmatokrit.

percentage. The longer column of blood will not only secure greater accuracy in reading the results, but also enables one to determine, with almost equal certainty, the volume of white corpuscles which, owing to their relatively lower specific gravity, form a second white column on the inner side of the red corpuscles.

The selection of a diluting liquid required much thought, time and labor. It becomes at once evident that this fluid should possess the following properties:

(1) It should absolutely prevent coagulation.

(2) It should preserve the normal shape of the corpuscles.

(3) It should harden the corpuscles so that no rupture occurs while rotating.

(4) It should give a *constant* volume in the *shortest* time.

(5) It should give the *largest* volume, so insuring greater accuracy in reading the result.

(6) It should possess a contrast color to that of the glass pipette, so facilitating and rendering more accurate the measuring of the diluting liquid.

(7) It should have no deleterious effects upon the white corpuscles.

(8) It should be of simple composition and form a permanent solution.

In experimenting with different liquids, blood from the same individual was taken, usually at the same hour, between 10 A.M. and noon, and afterward the blood that had been rotated was examined microscopically.

In determining the time required to obtain a fixed volume, observations were made every thirty seconds for ten minutes.

After a series of experiments lasting nine days, during which time nineteen different solutions were examined, I proved that a two and one half per cent. solution of bichromate of potassium fulfilled these conditions.

The solution possesses all the attributes of an ideal diluting fluid, namely:

(*a*) Absolutely preventing coagulation.

(*b*) Preserving the normal shape of the corpuscles.

(*c*) Hardening and so preventing rupture of the corpuscles.

(*d*) Giving a constant volume in sixty-six to seventy seconds.

(*e*) Giving the largest volume, thus diminishing the error in reading from the scale.

(*f*) Possessing a dark yellow color, contrasting strongly with that of the glass pipette, thus enabling one to measure the liquid with accuracy and ease.

(*g*) Staining the red cells so that the division between the column of red and white corpuscles is plainly seen.

(*h*) Apparently hardening the leucocytes.

(*i*) Making an extremely simple and stable solution, only requiring to be well corked to protect it from evaporation.

Lastly, an experience of four months, not only of myself, but also of my colleagues in the laboratory, *proves it to be by far the most useful liquid to dilute the blood for counting red blood corpuscles.*

A careful study was then made to determine the minimum amount of time required to secure a constant volume of red blood corpuscles, and after thirty observations it was shown that 100 revolutions of the larger wheel, or 10,000 revolutions of the frame carrying the tubes containing the blood, were amply sufficient when the 2·5 per cent. solution of potassium bichromate was employed. When the larger wheel was rotated 100 times rapidly, it required sixty-six to seventy seconds. Further rotation for one, two or more minutes was rarely successful in producing a reduction of one-fourth of a volume. In cases of extreme *leucocytosis* 200 rotations were found necessary, probably due to the relatively very light specific gravity of the white corpuscles.

In making a volumetric examination of the blood, the following articles are necessary:

(1) Alcohol.

(2) Ether.

(3) Large pin or a small lancet.

(4) Capillary pipette drawn to a point with a rubber tube.

(5) 2·5 per cent. solution of bichromate of potassium.

(6) Small watch-glass.

(7) Rubber tube to be attached to the glass tube of the hæmatokrit.

The lancet is first cleaned and then disinfected by the use of alcohol. The thumb of the left hand is most convenient. It should be thoroughly washed with ether and then allowed to dry, care being taken that no filaments from the towel remain. After making the incision, gentle pressure, causing the wound to gap, was usually found sufficient to produce a drop of blood the size of a pea; this was drawn into the pipette by suction, and an equal quantity of the bichromate solution added and thoroughly mixed in a

watch-glass. The hæmatokrit tube was then *immediately* filled by suction, one finger being held over the free end to prevent the displacement of blood, which would otherwise occur upon removal of the rubber tube. The filled tube is then placed in the frame of the hæmatokrit, and also a second prepared exactly in the same manner. The larger wheel is then rapidly rotated 100 times, and the results read from the scale, multiplied by 4, give the pecentage volume. The whole procedure should be done as *quickly* as possible, and particular care should be taken, as soon as the blood is mixed, that it be immediately rotated, otherwise the results obtained will indicate a larger volume of blood than really exists. The increased volume noticed upon allowing the mixed blood to remain in the watch-glass for a few minutes is probably due to the settling of the red blood corpuscles to the bottom because of their comparatively high specific gravity, and also to evaporation.

Having determined upon the method of pursuing the examination of blood by the hæmatokrit, it became important to determine the normal volume and its variations. For this purpose *thirty* healthy medical students, with an average age of twenty-four, were examined, and the percentage volume varied from sixty-two to forty-four, averaging fifty-one per cent.; a similar observation upon eight female nurses showed a variation of from forty-two to thirty-six, averaging forty-four per cent., or seven per cent. less than in the males. As the blood-counting apparatus is considered the best method of determining the number of red and white blood corpuscles, an attempt was made to show their relative accuracy, and also the probable number of corpuscles for each percentage volume. To accomplish this, the blood from twenty-five healthy men, physicians and students, was examined for the percentage volume, and at the same time a count was made with the following result: The average age was twenty-six years; the maximum volume observed was sixty-six per cent., and the minimum forty-four per cent., and an average of all the cases examined gave 51·8 per cent. volume. The red blood corpuscles in each case were carefully counted, and for this purpose the Thoma-

Zeiss hæmacytometer was employed. The average of all the counts made gives 5,088,442, and this being the case, one per cent. of volume is the equivalent of 98,578 red blood corpuscles. The difference between 99,390 and 100,000 is but 610; and, as in disease the average volume is reduced to 33·5, the greatest possible difference is but 20,435, a number so small as to be safely considered of no practical importance. In my opinion, therefore, for convenience, *one percentage volume may be considered as representing 100,000 red corpuscles.* This number greatly facilitates the calculating of the number of corpuscles present. When the percentage volume has been obtained, all that is necessary is to add five ciphers, and the number of corpuscles are indicated.

It is extremely interesting and important to note that the average volume of the thirty cases examined gave 51·5 per cent., and that this series of twenty-five gave 51·8 per cent., a difference of ·3 per cent., or 29,361 red blood corpuscles. These two series of observations, numbering in all fifty-five cases, prove most conclusively, first, that the average physiological volume for young men about twenty-six years of age is 51·6 per cent., and further, the fact that the average volume in both series speaks well for the *accuracy* of this method. With reasonable care the error by this method should fall within four volumes, or, roughly speaking, should not exceed 400,000; and when the modified apparatus here shown is adopted, it is quite certain that this working error may be reduced fifty per cent. or more, so that one may confidently expect that the volumetric reading shall fall within 200,000 of the true number of corpuscles, a deviation from normal so slight as to be of no practical importance.

From my own personal experience, and that gathered from my colleagues regarding the results obtained by the use of the Thoma-Zeiss hæmacytometer, a difference of two-, three-, five-hundred thousand or more is not at all unusual.

Reinert has clearly shown that this instrument gives a *theoretic* error of three per cent., or 150,000, in the hands of experts, and in *practical* clinical work, as will be proved later, it is certain that this error is considerably increased.

In making these observations, an effort was made to determine the normal volume of white corpuscles, but the extremely narrow white band, scarcely a line in width, renders accurate observations extremely difficult and often impossible. It was noticed, however, that in health the column of white corpuscles was never wider than one of the division lines cut in the glass tube. Any *considerable* increase in the white corpuscles is at once detected. The glass tube is modified, as here shown (see *Figs. 4* and *5*), so that the diameter of the lumen is decreased and the length doubled, permits of a more satisfactory study of the white corpuscles.

An analysis of the series of twenty-five cases referred to shows that the average age was twenty-six years ; that the maximum volume observed was sixty-six per cent., and the

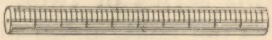

FIG. 4.—Original capillary tube, exact size.

FIG. 5.—Author's modified tube, showing its *exact* size and precise appearance of the scale.

minimum forty-four per cent., and an average of all the cases examined gave 51·8 per cent. volume. The red blood corpuscles in each case were carefully counted, and for this purpose the Thoma-Zeiss hæmacytometer was employed. The average of all the counts made gives 5,088,442, and this being the case, one per cent. of volume is the equivalent of 98,578 red blood corpuscles.

With this improved hæmatokrit one may make an examination in from five to ten minutes and determine at a *glance* the *volume* and *number* of *red* and *white* corpuscles.

In many of my cases the volume and number of corpuscles do not agree when five ciphers are added to the percentage volume, and this may be explained:

(1) By the variable results obtained, especially when we make but one preparation, and count sixty-four squares, as was done in the majority of the cases reported, and

SHOWING THE VOLUME AND NUMBER OF RED AND WHITE CORPUSCLES.*

Number of Case.	Age.	Sex.	Volume of White Corpuscles.	Volume of Red Corpuscles.	Number of Revolutions.	Hæmaglobin Fleischl Test.	Number of White Corpuscles.	Number of Red Corpuscles.	Probable Number of Red Corpuscles as Estimated from the Volume.	Diagnosis and Remarks.
						per cent.				
1	27	F.	1	42	10,000	5·4 or 38	6,783	3,220,000	4,200,000	Diabetes with typhoid fever.
2	34	M.	1	30	10,000	7·4 or 53	10,150	3,150,000	3,000,000	Hepatic cirrhosis. Internal jaundice. Blood looked red and watery.
3	20	M.	1	40	10,000	11·2 or 80	6,433	5,950,000	4,000,000	Acute croupous pneumonia. Third stage.
4	20	M.	1	44	10,000		7,155	5,775,000	4,400,000	Same case two days later.
5	21	M.	2	54	20,000	11·9 or 85	19,803	5,975,000	5,400,000	Acute croupous pneumonia. Second stage.
6	21	M.	2	48	20,000	—	13,630	5,463,000	4,800,000	Same case two days later.
7	33	F.	1	12	10,000	—	6,210	1,450,000	1,200,000	Gastric carcinoma. Intense anæmia. Blood looked pink.
8	65	M.	½	12	10,000	1·4 or 10	3,203	600,260	1,200,000	Essential anæmia. Blood pink and watery, and bled freely from puncture.
9	65	M.	½	8	10,000	10	3,900	562,500	800,000	*Ibid.* Diagnosis confirmed by autopsy.
10	43	M.	4	40	20,000	—	28,523	4,962,500	4,000,000	Acute croupous pneumonia. Second stage.
11	43	M.	1	44	10,000	80	9,050	5,437,500	4,400,000	*Ibid.* Convalescent and afebrile.
12	17	M.	2	32	10,000	—	15,414	5,375,000	3,200,000	Acute rheumatic pericarditis.
13	17	M.	2	36	10,000	—	18,471	4,720,000	3,600,000	*Ibid.*
14	17	M.	2	36	10,000	7·7 or 55	18,662	4,412,000	3,600,000	*Ibid.*
15	19	F.	1	30	10,000	7·7 or 55	8,340	4,150,000	3,000,000	Chronic endocarditis. Acute pleurisy. Temperature 38° C.
16	58	M.	1	28	10,000	—	8,057	3,181,808	2,800,000	Gastric carcinoma.
17	46	M.	1	24	10,000	—	7,070	3,593,750	2,400,000	Chronic peritonitis with ascites.
18	33	M.	1	20	10,000	—	12,102	2,230,000	2,000,000	Intestinal hemorrhage.
19	28	F.	3	50	20,000	—	24,000	5,250,000	5,000,000	Fourth day of acute croupous pneumonia of right lower lobe.
20	28	F.	3	48	10,000	—	22,484	5,787,500	4,800,000	*Ibid.* Eleventh day of the disease. No fever.
21	70	F.	2	18	10,000	35	32,000	2,712,500	1,800,000	Chronic interstitial nephritis confirmed by autopsy.
22	46	M.	4	28	20,000	45	32,248	3,637,500	2,800,000	Malignant sarcomatosis confirmed by autopsy.
23	15	M.	1	17	20,000	30	1,719	2,062,800	1,700,000	Lymphatic and splenic pseudo-leukæmia.
24	15	M.	½	16½	10,000	—	2,102	2,160,000	1,650,000	*Ibid.*
25	15	M.	½	16	10,000	30	4,012	2,187,500	1,600,000	*Ibid.*
26	21	F.	1	42	10,000	s —	11,783	5,962,500	4,200,000	Acute Bright's disease.
27	38	F.	1	33	10,000	s —	9,936	4,410,000	3,200,000	Pregnancy.
28	45	M.	1	36	10,000	s 55	3,503	4,956,000	3,600,000	Tertian intermittent fever.
29	36	F.	2	36	10,000	s —	17,006	5,237,000	3,600,000	Acute myelitis.
30	29	F.	13	20	20.000	s 40	330,956	3.025,000	2,000,000	Splenic leukæmia.
31	29	F.	13	24	20,000	s 40	275,000	2,900,000	2,400,000	*Ibid.*
32	29	F.	13	25	20,000	s 40	181,250	3,160,000	2,500,000	*Ibid.*
33	29	F.	13	22	20,000	s —	180,000	3.575,000	2,200,000	*Ibid.*
34	29	F.	1	33	10,000	s —	6,496	3.470,969	3,300,000	Tumor cerebri syphilitic.
35	20	M.	2	36	20,000	s 65	16,123	4,830,000	3,600,000	Acute articular rheumatism.
36	20	M.	2	42	20,000	s —	7,126	4,830,000	4,200,000	*Ibid.* Articular rheumatism.
37	33	M.	2	48	20,000	s 12·6 or 90	13,695	5,864,000	4,800,000	Perityphlitis, afebrile.
38	21	M.	1	50	10,000	—	—	5,037.500	5,000,000	Chlorosis.
39	21	M.	1	52	10,000	55	—	5,387,500	5,200,000	*Ibid.*
40	67	M.	1	30	10,000	s 65	7,197	3,974.500	3,000,000	
41	23	F.	1	27	10.000	35	6,170	3.200,000	2,700,000	Chronic parenchymatous nephritis.
42	27	F	1	38	10,000	—	4,108	4,637,500	3,800,000	Simple anæmia.
43	24	M.	1	38	10,000	—	—	3,937,000	3,800,000	Simple anæmia.
44	40	M.	2	70	10,000	102	12,172	6,912,000	7,000,000	Cholera nostra.

* The counts here given made by my colleague, Dr. Carl Sadler, are marked "s."

(2) That the blood used to determine the volume was always mixed with the bichromate of potassium solution in the ampulla of the Zeiss pipette, and not separately measured and mixed in a watch-glass; and therefore, in all cases where the tendency to coagulation was increased, the volume was eight or more per cent. less than really existed.

From the studies which I have here recorded, the following opinion is deduced *that the hæmatokrit gives as accurate, if not more accurate, results than the Thoma-Zeiss apparatus as ordinarily employed, requires less skill, calls for no eye-strain, and the volume of red blood corpuscles, and number per cubic millimetre, and volume of white corpuscles, may be determined within ten minutes.*

In conclusion, I beg leave to append the following table, showing variation in the volume of the blood cells in a variety of diseases :

(a) That the fluid used to determine the volume was always mixed with the bichromate of potassium solution in the amount of the [illegible] pipette, and the mixture [illegible] and mixed in a watch-glass and [illegible] where the tendency to coagulation was increased, the volume was [illegible] than usual [illegible].

From the [illegible] which I have here recorded, the following conclusion is [illegible] [illegible] [illegible].

In conclusion, I beg leave to append the following table, showing variations in the volume of the blood cells in a variety of diseases: